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Communication

[*Comunicação*]

Chromobacterium violaceum associated with bubaline mastitis

[*Mastite bubalina por* Chromobacterium violaceum]

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Buffalo mastitis is a disease that causes inflammation of the mammary gland (Viguier *et al*., 2009). The infectious mastitis is the most prevalent, resulting in low milk production in buffaloes (Keefe, 2012) and significant economic losses for the producer (Carvalho *et al.*, 2007), as well as being a risk to public health, due to the high potential for transmission of pathogens from milk to the population (Vliegher *et al.*, 2012).

Multiple infectious agents can cause mastitis disease in buffaloes, including bacteria (Viguier *et al*., 2009). The most prevalent bacterial agents in milk of buffaloes with mastitis are gram-positive, *Staphylococcus* spp., *Corynebacterium* spp., and *Streptococcus* spp., and gram-negative such as *Escherichia coli* (Medeiros *et al.*, 2013). However, bacteria that are unusual for this infectious disease can also affect buffaloes and cause mastitis disease, for example, the bacterium *Chromobacterium violaceum* (*C. violaceum*). During the writing of this short communication, no report of mastitis in buffaloes caused by *C. violaceum* bacteria was found in the literature.

C. violaceum is a natural bacterium from water and soil of regions with tropical and subtropical climates (Han *et al*., 2008; Bittencourt *et al.*, 2011) it can infect animals and humans that come into contact with the contaminated environment (Desjardins *et al.*, 1999). It has low morbidity, high mortality, and high lethality in animals (Steinberg and Burd, 2010) and humans (Ajithdoss *et al.*, 2009; Chou *et al*., 2000).

Infections with *C. violaceum* in animals were first described in 1905, causing sepsis in buffaloes in the Philippines (Woolley, 1905). Subsequently, infections have been reported in other production animals, as examples: pigs, causing diarrhea, dyspnea, and pleuropnemonia (Liu *et al*., 1989); sheep, causing dyspnea and pleuropnemonia (Carrasco *et al*., 1996); equine, generating intense apathy, fever (41.3°C), severe dyspnea and pulmonary insufficiency, caused by sepsis (Hammerschmitt *et al*., 2017); bovine, causing omphalophlebitis, polyarthritis, uveitis, and encephalitis, a clinical condition resulting from severe sepsis (Soares *et al*., 2019).

In humans, it was first described in Malaysia, in 1927, in a patient with liver abscesses and fatal sepsis (Ponte and Jenkins, 1992). *C. violaceum* infections in humans are considered rare and occasionally fatal and can promote, among other symptoms, hyperthermia, abdominal pain (Laghu *et al*., 2021), changes in the lungs, liver, and spleen (Dias *et al*., 2005), urinary tract infection, and sepsis (Sachu *et al*., 2020). The most recent report of *C. violaceum* infection in humans in Brazil was in 2014. The fatal case was an 8-year-old female child, who arrived at the hospital in Natal, Rio Grande do Norte state, Brazil, presenting five days ago with fever, abdominal pain, and pharyngotonsillitis, dying four hours after hospitalization (Fernandes *et al*., 2014).

C. violaceum has been detected in different environmental areas distributed throughout the states of Brazil. For example, isolation in water and soil samples from a site located in a city in

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Baixo Sul, Bahia State (Dias *et al*., 2005); water samples collected from a fish farm located in Conceição das Alagoas, Minas Gerais State (Oliveira *et al*., 2017); and water samples from the mouth of Igarapé Grande and Igarapé Mirandinha belonging to Rio Branco, Boa Vista City, Roraima State (Farias *et al*., 2021).

Despite the low morbidity (Steinberg and Burd, 2010), its transmission in buffaloes happens due to the natural habit of buffaloes to stay for long periods in water and mud (Pathak, 1992), makes them susceptible to bacterial infections, causing in female buffaloes' mastitis and other pathologies (Garcia, 2006).

The increasing production of dairy buffaloes in Brazil, underreporting of bacteria that cause buffalo mastitis, risk of transmission of pathogens present in milk to the human and animal population, and economic losses in dairy farming, reinforce the importance of this communication to society, besides encouraging continuous and periodic studies on the health of the bubaline species, quality control that their products and byproducts are being offered to the market and knowledge about rare bacterial infections, strengthening the adherence of microbiological tests, such as lactoculture to identify the etiology of mastitis, and the antibiogram to identify the best drug to be used in treatments for this disease. Given the above, we report the occurrence of a case of buffalo mastitis caused by *Chromobacterium violaceum* bacteria.

In view of the increasing production of dairy buffaloes in Brazil, the underreporting mastitis-causing bacterial species in buffaloes, the risk of transmission of milk-borne pathogens to the population, and the economic losses in dairy farming, this study aims to report the first case of buffalo mastitis caused by *C. violaceum* in Brazil.

A Murrah female, 4 years old, 420kg, color black, milk production under 1,800 liters per lactation, presenting with subclinical mastitis and a history of recurrent subclinical and clinical mastitis. Breeding is carried out in rotational grazing systems using elephant grass-based diets with grain-based supplements, and access to water through troughs and dams. The property is in the semiarid of Pernambuco state, Brazil, with mechanized milking performed twice a day, with a total number of 87 buffaloes.

An amount of 4.0 mL of milk was collected from the four teats of the mammary gland in previously identified sterilized *Falcon* tubes. All the samples were transported to the Laboratory of Infectious-Contagious Diseases of Domestic Animals (LDIC) of the Federal Rural University of Pernambuco, Microbiology sector, under refrigeration (2°C a 4°C) in isothermal boxes containing recyclable ice for their processing.

The samples were cultured on base agar enriched with 7% of sheep blood and incubated at 37°C in aerobic conditions. Readings occurred at 24, 48, and 72 hours post-incubation (Koneman *et al*., 2008). A macroscopic analysis of the colonies was performed, and all of them were analyzed microscopically using the *Gram* stain method. Bacterial confirmation was completed using the *VITEK 2 - Compact*® system.

Antimicrobial susceptibility test was carried out using the disk diffusion method. For this, bacterial colonies were inoculated in a test tube containing 3.0 mL of Brain – Heart Infusion broth (BHI) and incubated at 37°C in aerobic conditions for 18 hours, to obtain turbidity equivalent to 0.5 of the *McFarland* standard scale. Subsequently, with the aid of sterile swabs, the inoculum was sowed in Petri dishes containing Mueller-Hinton agar (Performance…, 2005).

Antibiotic disks containing ceftiofur (30µg), ciprofloxacin (5µg), erythromycin (15µg), gentamicin (10µg), penicillin G. (30µg), sulfazotrim (25µg), and tetracycline (30µg) were used. The interpretation was made by measuring the inhibition zones after 18 hours of incubation (Performance…, 2005).

After 72 hours of incubation, only the sample obtained from the right anterior teat showed bacterial growth. Violet-colored colonies with beta-hemolysis, shiny and mucoid appearance, circular shape, smooth surfaced, and sizes ranging from 0.5mm to 1.0mm in length were noticed (**Fig.1**). Microscopically, *Gram*-negative bacilli (obj.100X) were observed. Confirmation of the species *C. violaceum* was carried out using the *VITEK 2 - Compact*® system.

Source: Own archive.

Figure 1. Colonies of *C. violaceum* on base agar enriched with sheep blood (7%). It can be observed violet-colored colonies with beta-hemolysis, shiny and mucoid appearance, circular shape, smooth surfaced, and sizes ranging from 0.5mm to 1.0mm in length.

The antibiotic susceptibility test showed that *C. violaceum* was sensitive to ciprofloxacin, gentamicin, sulfazotrim, and tetracycline and presented resistance to ceftiofur, erythromycin, and penicillin G.

The buffalo underwent intramammary antibiotic therapy using ciprofloxacin, associated with intramuscular gentamicin, showing a positive response during the treatment. In the same year, the buffalo presented new episodes of subclinical mastitis, and later, clinical mastitis, in none of the cases was the microbiological agent or other causes that were favoring the appearance of the disease identified. The study cannot be continued, including new lactocultures or other diagnostic tests for other diseases, due to the sacrifice of the buffalo.

In addition to recurrent clinical and subclinical mastitis, the buffalo presented a thickening of the anal sphincters that made palpation and pregnancy diagnosis impossible.

It was not possible to carry out more in-depth studies due to the sacrifice of the buffalo after the confirmation of *C. violaceum* infection. Lactocultures were carried out on other buffaloes on the property, but all were negative for infection by *C. violaceum*.

This is the first record of bubaline mastitis caused by *C. violaceum* bacteria in Brazil. It is believed that *C. violaceum* infection was caused due to the buffalo's habit of staying for long periods in water and soil, and the bacterium used the mammary gland to cause the infection because no traumas were found on the buffalo mammary gland, which would facilitate the access of the pathogen.

In the studied buffalo's herd, the characteristic of low morbidity was the same reported in the literature, since only one buffalo in the herd was diagnosed with *C. violaceum* infection. Similar considerations were reported by Steinberg and Burd (2010), when describing that animals rarely become infected with *C. violaceum*, however, once infected, cause severe and often fatal infectious conditions.

The antibiotics ciprofloxacin and gentamicin demonstrated efficacy *in vitro*, so they were selected for antibiotic therapy. Aldridge (1988) studied the sensitivity profile of *C. violaceum* strains to 25 antibiotics, noting that ciprofloxacin demonstrated greater effectiveness when compared to other tested antibiotics. In the same study, when aminoglycosides were tested, gentamycin was the one with the highest activity.

Ciprofloxacin and gentamicin also showed a sensitivity profile to a *C. violaceum* sample isolated from a 76-year-old woman who presented clinically with pustules on the skin, followed by sepsis and death (Sachu *et al*., 2020). Laghu *et al*. (2021) studied the sensitivity profile of *C. violaceum* from a sample from a 41-year-old male hospital patient presenting with fever, abdominal pain, and hematuria, the result was sensitivity to the antibiotic gentamicin.

These results show that the antibiotics ciprofloxacin, belonging to the quinolone class, and gentamicin, belonging to the aminoglycoside class, should be chosen as a priority for studies on the antibiotic sensitivity profile for the bacterium *C. violaceum* to combat cases of general infections in humans or in animals in cases of need.

The opportunistic bacterium *C. violaceum* has the characteristic of producing biofilms (Martinelli *et al*., 2002), in addition, some strains already studied and possibly other strains, have extrachromosomal DNA (Lima *et al*., 2018) that allow the characterization of intrinsic resistance to various antibiotics and high virulence, worsening prognoses and hindering possible treatments for infections caused in animals and humans.

Recently, the pChV1 plasmid was detected in the *C. violaceum* strain ATCC 12472, which indicates that some other strains of *C. violaceum* may harbor extrachromosomal DNA and, consequently, among other characteristics, confer resistance to some antibiotics (Lima *et al*., 2018). In another study carried out by Lima *et al*. (2021), six draft plasmid genomes were discovered in *C. violaceum* strains, as a result, at least three plasmids were similar to pChV1. Studies of this nature are important to understand the genomic and phylogenetic profile of this pathogen, and consequently, to understand the pathogenic characteristic of this microbiological agent and its resistance genes, helping to develop new genetic and biotechnological studies for this bacterium.

The buffalo entered the farm at the age of three and, according to the results of the disc diffusion tests performed on milk samples from some buffaloes on the property after its entry, there was no history of clinical or subclinical mastitis associated with other bacteria that resistance to the groups of antimicrobials tested is characterized. Some differences found in the sensitivity profile of *C. violaceum* suggest that the bacterium may have different resistance genes for some drugs tested due to its own genetic and evolutionary characteristics.

Reports of some cases of infections in animals and humans and environmental contamination described in the literature and presented in this article, together with information on the first case of buffalo mastitis caused by *C. violaceum*, demonstrate the importance of periodic studies on *C. violaceum* in aquatic environments and soils belonging to all states of the Federation of Brazil, as well as in water and milk intended for animal and human consumption, in order to prevent new infections and allow rapid diagnosis and effective treatment.

It should be noted that contaminated milk can be considered a transmission route for bacteria that commonly cause subclinical or clinical mastitis, or opportunistic bacteria, both for animals, especially during the breastfeeding period or milking, and for humans during ingestion of natural or its derivatives. It may also be a possible source of formation and transmission of multiresistant bacterial populations when an animal is subjected to treatment with antibiotics, but its withdrawal period is not respected, in addition to which, the milk, when discarded incorrectly in the environment, can also become source of environmental contamination or animal and human infection, causing serious consequences for unique health.

A case of buffalo mastitis caused by the bacterium *C. violaceum* is registered for the first time. It is suggested to adopt efficient hygienic-sanitary management in the farms and periodic microbiological investigations in buffaloes with and without an antecedent of mastitis. These measures will minimize the low productivity of the herd and reduce economic losses for the farmer, in the same way that they will avoid the infection and transmission of *C. violaceum*, and other pathogens present in milk to other animals and human population.

Keywords: buffaloes, lactoculture, antibiogram, economic losses, One Health

RESUMO

Objetivou-se com este estudo relatar a ocorrência de um caso de mastite bubalina ocasionada por Chromobacterium violaceum. *Foram coletados 4,0mL de leite de cada teto mamário de uma búfala da raça* Murrah. *As amostras foram semeadas em ágar base enriquecido com 7% de sangue ovino desfibrinado. As leituras foram realizadas 24, 48 e 72 horas pós-incubação. Apenas o teto anterior direito apresentou crescimento de colônias de coloração violeta-escura. Microscopicamente, observaram-se bacilos* Gram *negativos. A confirmação da espécie foi realizada pelo sistema* VITEK 2 - Compact®, *sendo identificada como* Chromobacterium violaceum. *Essa bactéria apresentou sensibilidade a ciprofloxacina, gentamicina, sulfazotrim e tetraciclina e resistência a ceftiofur, eritromicina e penicilina G. O tratamento foi realizado com ciprofloxacina e gentamicina, apresentando resposta positiva. Estudos dessa natureza reforçam a importância desse comunicado para a sociedade, além de incentivar pesquisas contínuas e periódicas sobre sanidade da espécie bubalina, controle de qualidade que seus produtos e subprodutos estão sendo oferecidos ao mercado e conhecimento sobre infecções bacterianas raras, fortalecendo a adesão de exames microbiológicos, como lactocultura e antibiograma. Diante do exposto, registra-se a ocorrência de um caso de mastite bubalina ocasionada pela bactéria* Chromobacterium violaceum.

Palavras-chave: búfalas, lactocultura, antibiograma, perdas econômicas, Saúde Única

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